Experimental Design (Bioassay Cholinergics)

Mouse Strain	Dose (μg)	No. of Animals	Measurement							
			1	2	3	4	5	6	7	8
AKR/J	Control	12								
	0.0	12								
	0.1	12								
	0.3	12								
	1.0	12								
	3.0	12								
	10.0	12								
	30.0	12								
	100.0	12								
C57BL/6J	Control	12								
	0.0	12								
	0.1	12								
	0.3	12								
	1.0	12								
	3.0	12								
	10.0	12								
	30.0	12								
	100.0	12								

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BIOASSAY FOR MEASUREMENT OF CHOLINERGIC AND ANTICHOLINERGIC DRUG POTENCIES IN MICE*

by

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Cambridge, Massachusetts

* Paper presented at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics, April 12-17, 1970, Atlantic City, N.J.

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BIOASSAY FOR MEASUREMENT OF CHOLINERGIC AND ANTICHOLINERGIC DRUG POTENCIES IN MICE.*

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Mr. Chairman, members of the Society:

We reported previously on a nontraumatic method for the quantitative measurement of salivation in minimally restrained unanesthetized mice.

Narrow cotton fabric strips, pretreated with cresol red indicator, are inserted into the mouth by means of an oral wire bit to absorb saliva. The length of the diffused colored band, visible after a given time interval, is measured in mm, and indicates the amount of saliva excreted. Immobilization of the mice for measuring salivation is achieved by fitting "collar" around the neck of the animals and inserting the collar into a "stanchion" during measurements. Such an arrangement permits the simultaneous study of salivation in a large "herd" of mice.

Slide I shows a mouse in a stanchion by its permanently worn collar. The mouse bites on a wire "bit." A narrow cotton strip, attached to the bit turns from yellow to purple upon exposure to saliva.

Next slide shows that a large number of mice can be studied simultaneously. Significant differences in salivary performance exist among various strains of mice.

High salivating C57BL mice, low salivating AKR/J mice, all supplied by the Jackson Laboratories in Bar Harbor, Maine.

It is the purpose of this paper, first to describe the immediate and aftereffects of two drugs on salivary performance of AKR and C57BL mice (the two drugs are methacholine chloride and atropine sulfate), second, to examine the feasibility and to determine the optimal conditions of this experimental approach as a bioassay for the measurement of cholinergic and anticholinergic drug potencies.

The next slide presents the experimental layout to study the effect of methacholine on salivation. A similar experimental design was followed for atropine. Male AKR and C57BL mice were used. Salivation of the

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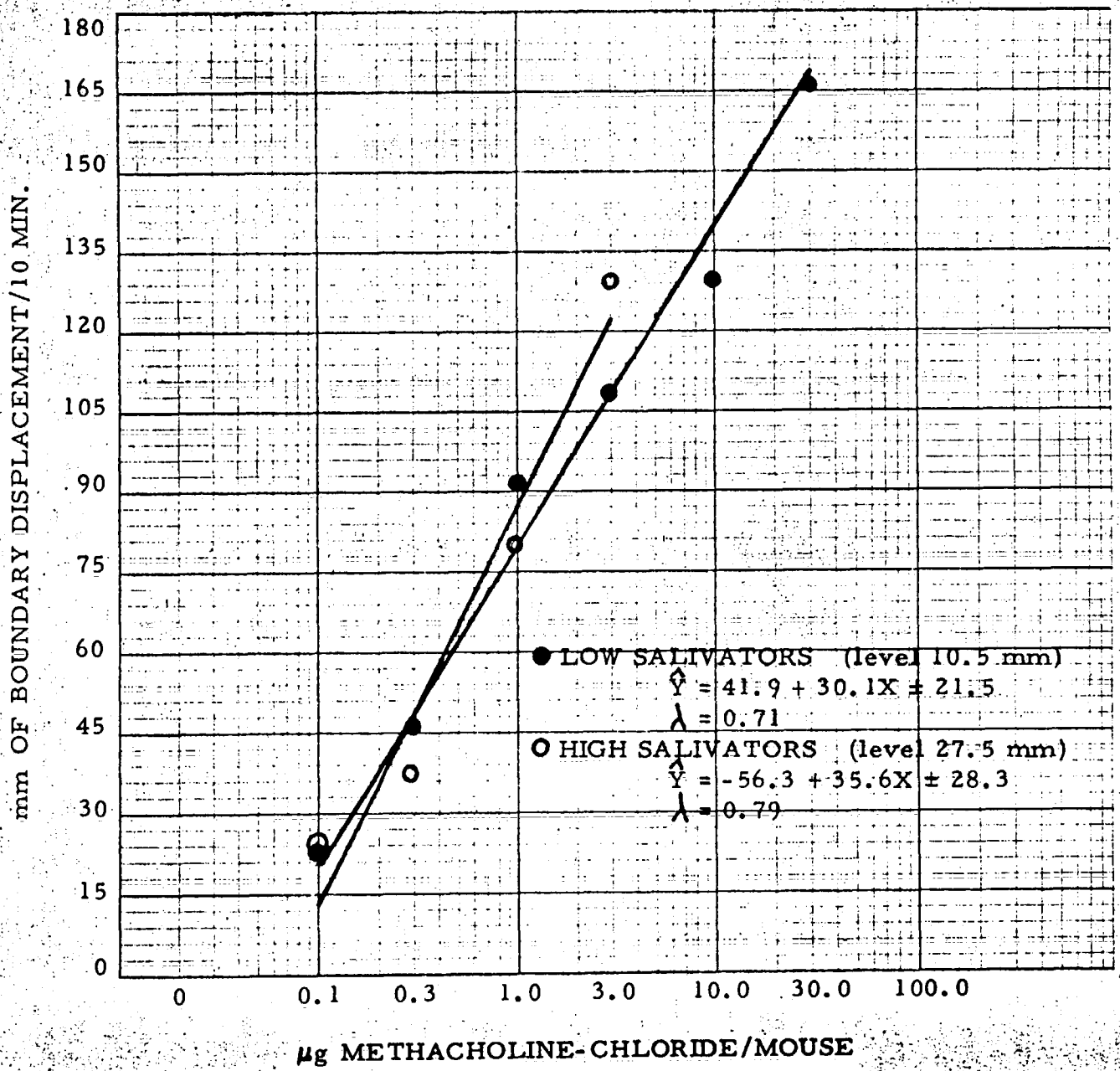
RELATIONSHIP BETWEEN A SERIES OF DOSES OF METHACHOLINE CHLORIDE AND IMMEDIATE SALIVATION IN MALE AKR/J MICE PRESELECTED FOR HIGH AND LOW SALIVARY PERFORMANCE (LINEAR PORTION ONLY)



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Characteristics of the Dose-Response Relationship between a Logarithmic Series of Doses of Methacholine Chloride and the Immediate Salivation in Two Strains of Male Mice with Varying Salivary Performance (Linear Portion Only)

	Threshold (μg)	Linear Range (μg)	r	Regression $\hat{Y} = a + bX \pm S_{yx}$	λ
C57BL/6J					
high	< 0.1	0.3- 3.0	0.43	31.5+18.8X±34.7	1.85
low	< 0.1	0.3- 3.0	0.74	-69.2+39.8X±31.0	0.78
AKR/J					
high	< 0.1	0.1- 3.0	0.84	-56.3+35.6X±28.3	0.79
low	< 0.1	0.1-30.0	0.91	-41.9+30.1X±21.5	0.71

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The experimental design can be arranged to study both the immediate and aftereffects effectively, and data can be obtained simply and in adequate numbers to permit statistical analysis of results.

Methacholine was found to stimulate salivation initially, followed by a salivary inhibition. Atropine, on the other hand, only showed salivary inhibition. These effects are dose-dependent.

The immediate effects of methacholine and of atropine were explored for bioassay purposes. Optimal conditions for the bioassay of methacholine require the use of low-salivating individuals from a low-salivating strain of mice. For the bioassay of atropine, one should use high-salivators from a high-salivating strain of mice.

The threshold dose of each drug is smaller than 0.1 μg; the linear dose range is at least one hundred; and the indices of precision are acceptable.

In conclusion, this new approach which uses the salivation of mice, is suitable for bioassay purposes of cholinergic and anticholinergic substances.

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mice was measured eight times during one day at intervals of one hour. Measurements 1 and 2 served as controls to establish the salivary performance of individual mice. These salivary performances then served as a basis for randomization of the mice into one of nine treatment groups. Immediately prior to the third measurement, drug dosages were applied. Thus the third salivation measurement indicates the immediate drug effect. Measurements 4 through 8 were included to quantitate any aftereffects to the drug.

The nine treatments applied were "control," "water" and a logarithmic series of doses of methacholine, ranging from 0.1 through 100 μg, injected subcutaneously in 0.1 ml of water.

It is of interest to point out that any drug treatment can thus validly be compared with two controls, namely first with the "control" and "water" treatment groups, but second also with their own salivation performances during the first and second measurements, as earlier results had shown that no significant differences in salivation exist during the day.

Part of the data will now be presented to illustrate the overall salivation pattern in two strains of mice, caused by methacholine or atropine.

The next slide shows the effect of 0.3, 3.0 and 30 μg methacholine on the salivary performance of high salivating C57BL mice. Note that methacholine stimulates salivation initially and mice over a relative small dose range (from a control level of 60 to 130 mm for 30 μg of methacholine). The salivary stimulation is afterwards changed to a salivary inhibition.

The next slide shows the same treatments of methacholine on salivation of low-salivating AKR mice. Note that the salivary increase is much more pronounced (from a control level of 15 to 160 mm with 30 μg of methacholine).

The next slide shows the effect of atropine on salivary performance of low-salivating AKR's. There is a relative small salivary decrease (from control level of 30 to 5 mm with 30 μg of atropine).

The next slide shows the salivary inhibition by atropine in high-salivating C57BL mice. Obviously, this inhibition from a control level of 47 to 3 mm with 30 μg of atropine, is much more dramatic than that shown in the previous slide with AKR mice.

The foregoing results suggest that it is feasible to use the salivary stimulation of a low-salivating strain, and the salivary inhibition of a high-salivating strain of mice for bioassay purposes.

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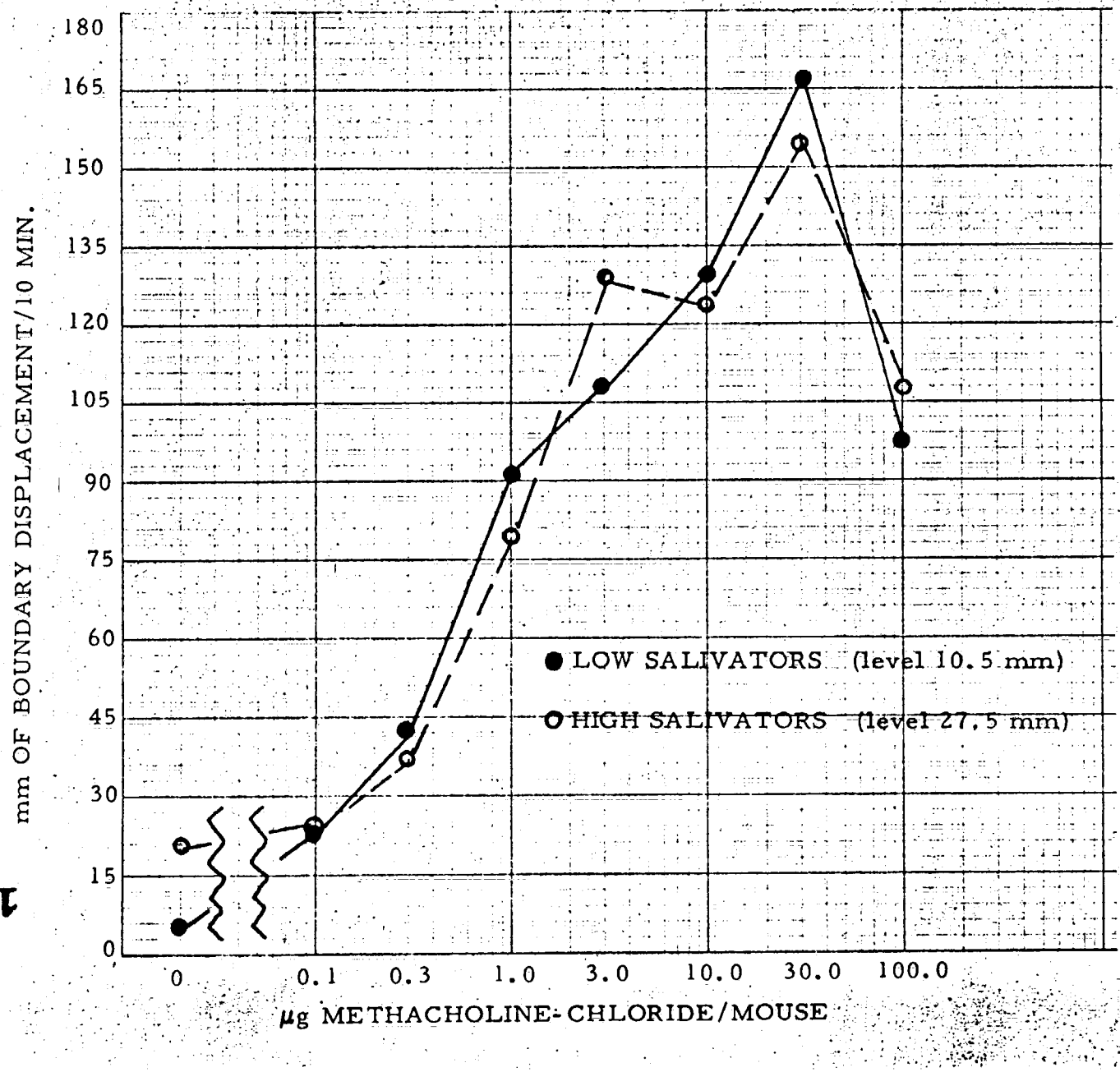
RELATIONSHIP BETWEEN A SERIES OF DOSES OF METHACHOLINE CHLORIDE AND IMMEDIATE SALIVATION IN MALE AKR/J MICE PRESELECTED FOR HIGH AND LOW SALIVARY PERFORMANCE



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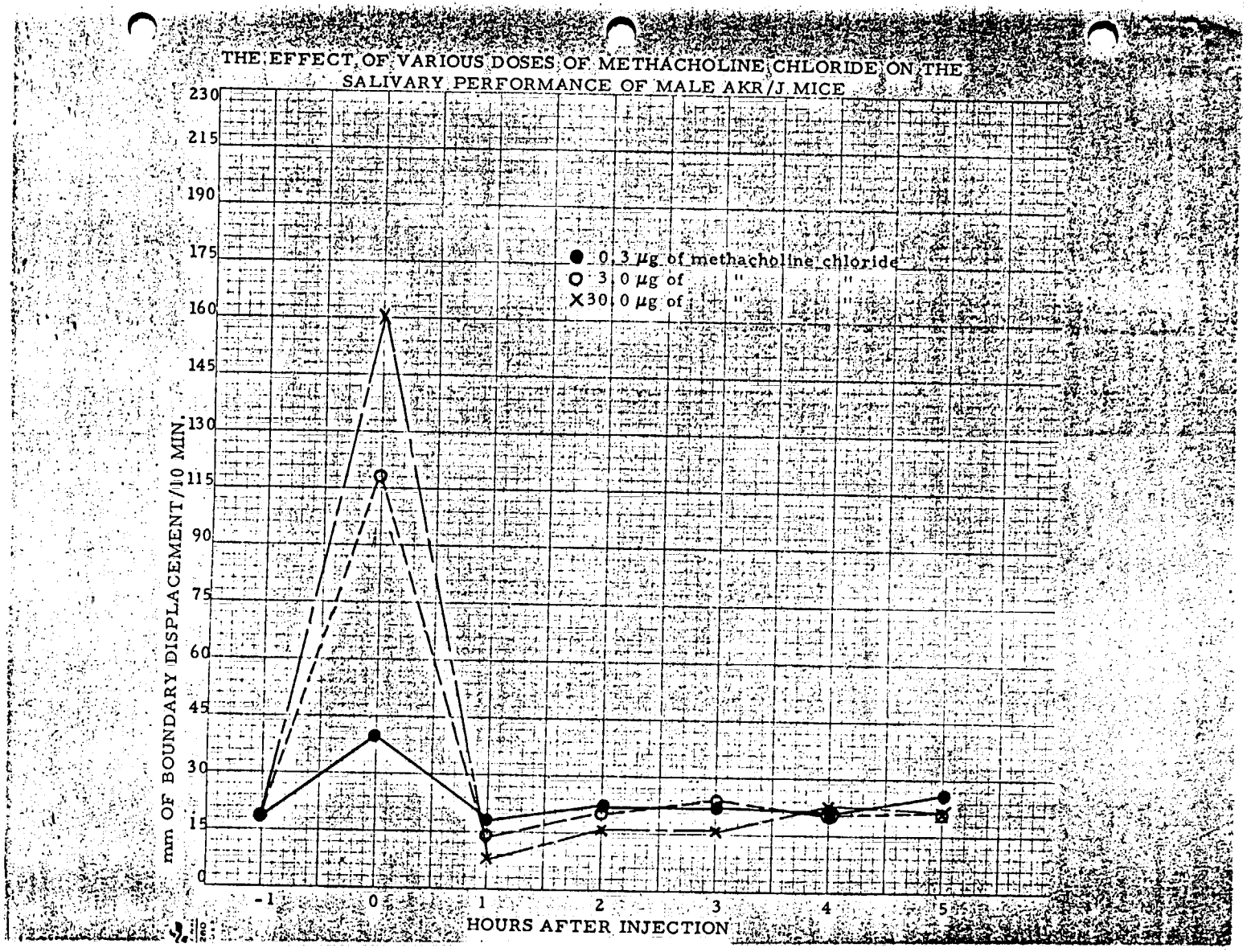

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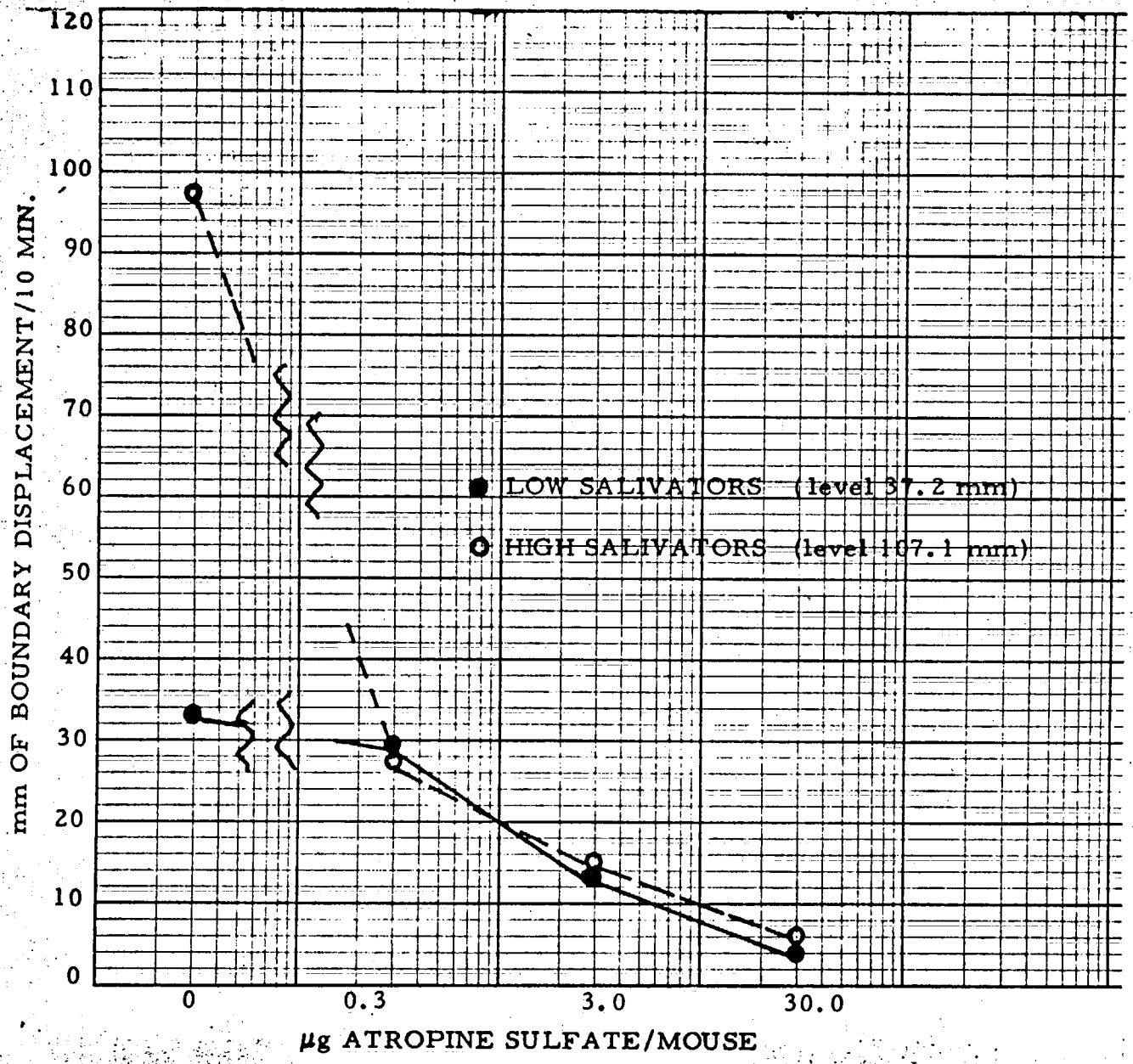
RELATIONSHIP BETWEEN A SERIES OF DOSES OF ATROPINE SULFATE AND IMMEDIATE SALIVATION INHIBITION OF MALE C57BL/6J MICE PRESELECTED FOR HIGH AND LOW SALIVARY PERFORMANCE



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We will now examine in some detail the immediate responses of methacholine on salivation of AKR's and of atropine on the salivation of C57BL mice.

The next slide shows the relationship between a series of doses of methacholine and immediate salivation in male AKR mice. The experimental animals were separated into high and low salivating individuals within the AKR strain. A good dose-response relationship is noticeable with each group over the dose range of 0. through 30 μg of methacholine.

The next slide shows the calculated linear regressions and indices of precision over the 0.1-30 μg dose range. The indices of precision are 0.79 and 0.71, respectively, indicating a slight improvement of the method through application of preselection of individuals within the AKR strain.

The next slide summarizes the relative usefulness of high and low-salivating C57BL, and the high and low-salivating AKR/J for bioassay purposes of methacholine. The threshold of methacholine is below 0.1 μg for each, but the linear dose range, as well as the indices of precision show that low-salivating AKR's are superior to any other mice.

The next slide shows the relationship between a series of doses of atropine and immediate salivation inhibition of male C57BL mice. Here again, the experimental animals were separated into high or low-salivating individuals within the C57BL strain. A good dose-response relationship can be seen over the range 0.3 through 30 μg of atropine.

The next slide shows the calculated linear regressions over the same dose range. Although the slopes are smaller than those of the D - R curves with methacholine, the variability is also smaller. This is indicated by the favorable ' of 0.45 and 0.53, respectively.

The next slide summarizes the relative usefulness of high and low-salivating C57BL and high and low-salivating AKR's for bioassay purposes of atropine. The best index of precision was indeed obtained with preselected high-salivating C57BL mice.

In summary

A new experimental approach has been presented for the measurement of salivation in rodents. The method employs minimally restraint unanesthetized mice, and is suitable for pharmacological studies on salivation.

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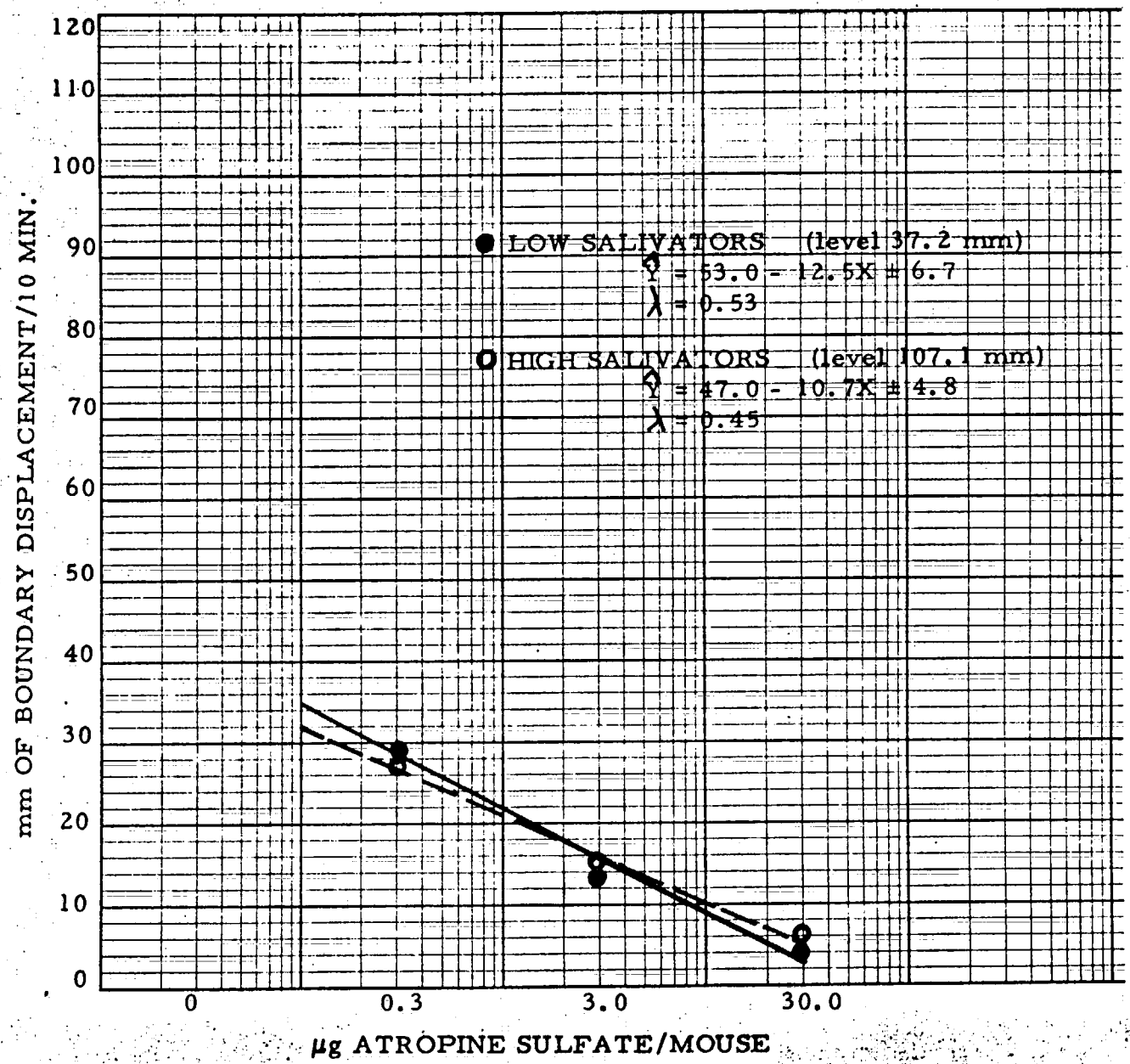
LINEAR REGRESSION BETWEEN A SERIES OF DOSES OF ATROPINE SULFATE AND IMMEDIATE SALIVATION INHIBITION IN MALE C57BL/6J MICE PRESELECTED FOR HIGH AND LOW SALIVARY PERFORMANCE



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Characteristics of the Dose-Response Relationship between a Logarithmic Series of Doses of Atropine Sulfate and the Immediate Salivary Inhibition in Two Strains of Male Mice Preselected for High and Low Salivary Performance

	Threshold (μg)	Linear Range (μg)	r	Regression $\hat{Y} = a + bX \pm S_{yx}$	λ
C57BL/6J					
high	< 0.1	0.3-30	0.89	47.0-10.7X± 4.8	0.45
low	< 0.1	0.3-30	0.85	53.0-12.5X±6.7	0.53
AKR/J					
high	< 0.1	0.3-30	0.52	33.1- 6.4X±9.3	1.45
low	< 0.1	0.3-30	0.50	23.3- 3.9X±5.9	1.51

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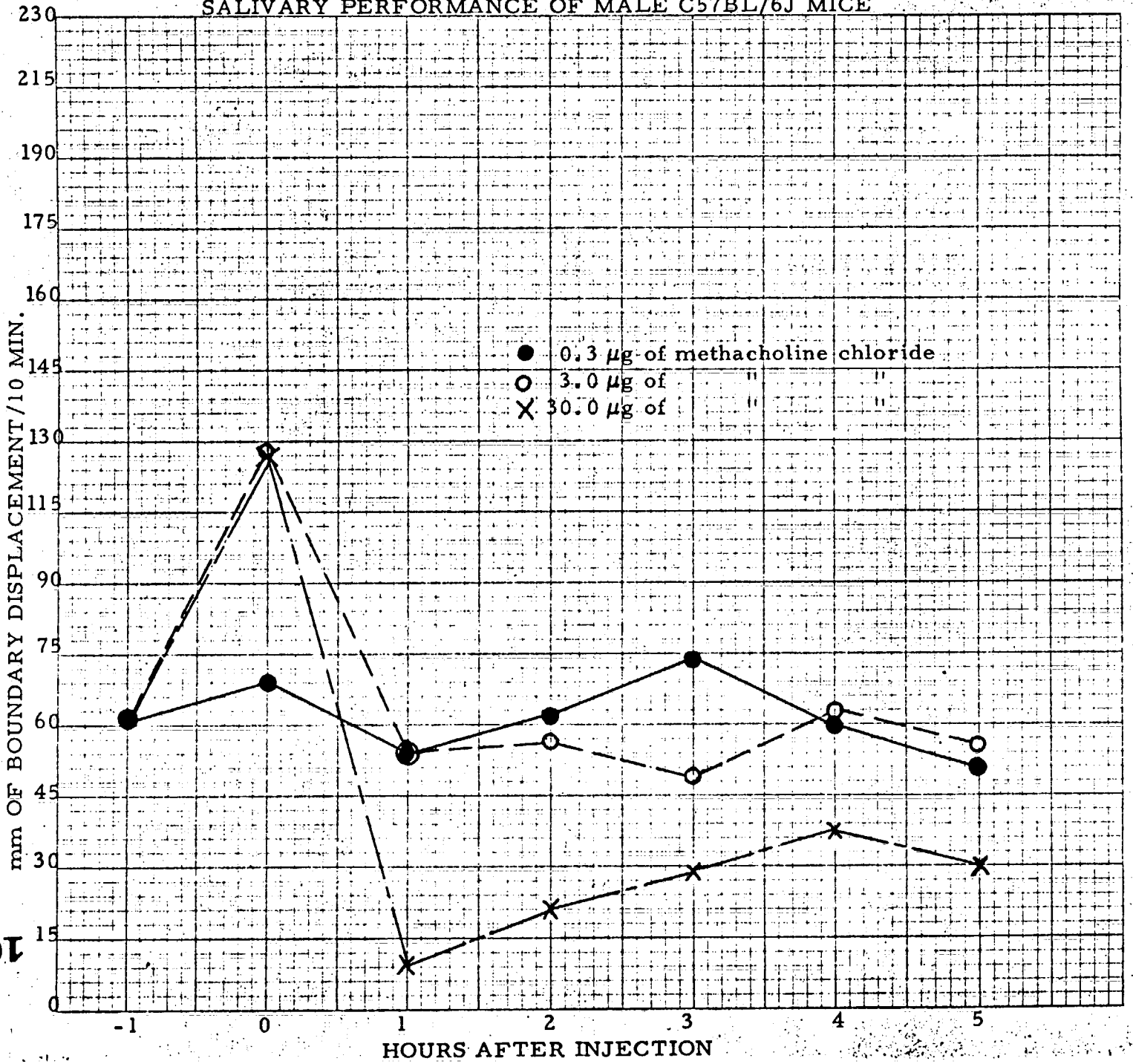
THE EFFECT OF VARIOUS DOSES OF METHACHOLINE CHLORIDE ON SALIVARY PERFORMANCE OF MALE C57BL/6J MICE



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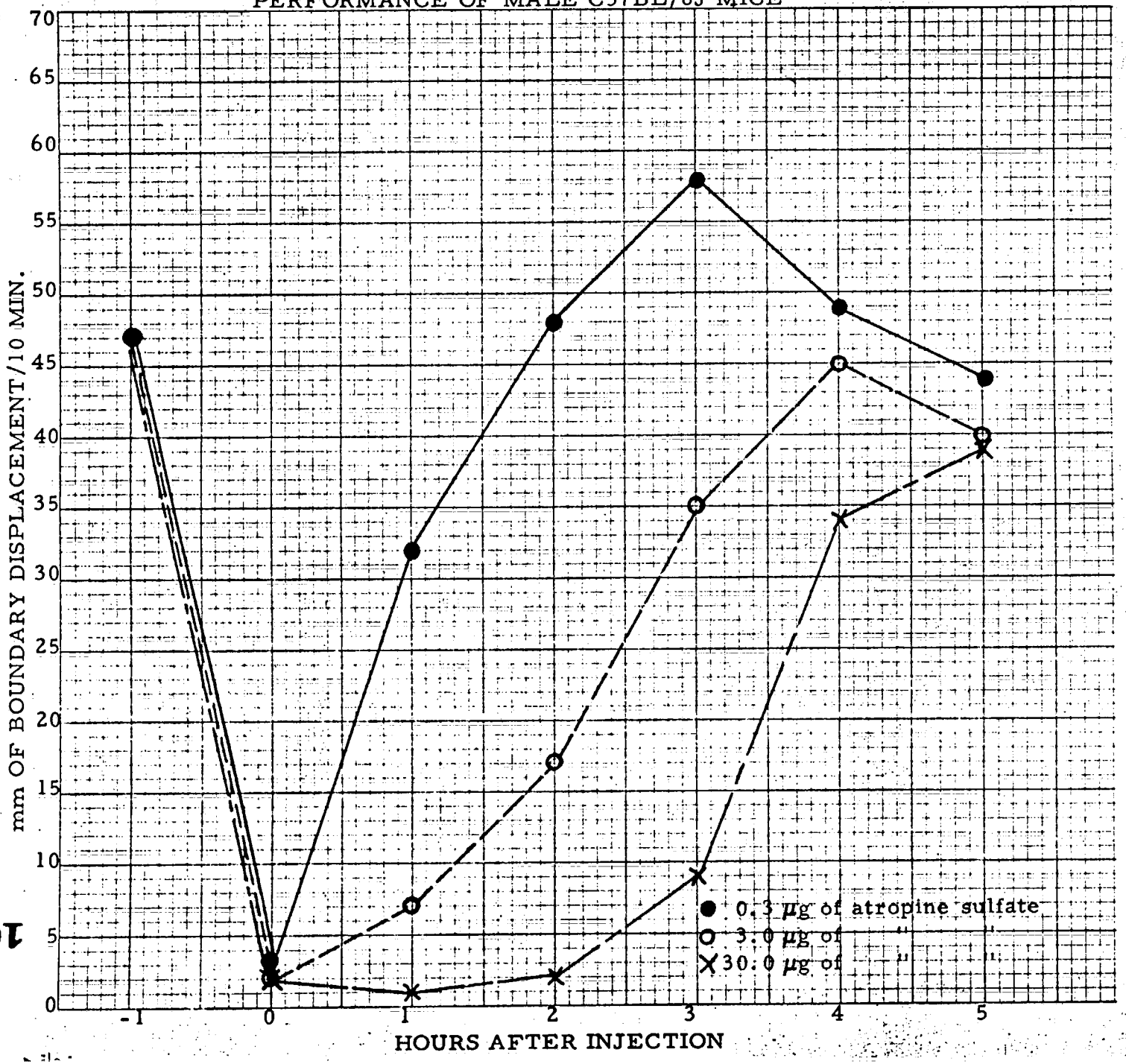
THE EFFECT OF VARIOUS DOSES OF ATROPINE SULFATE ON SALIVARY PERFORMANCE OF MALE C57BL/6J MICE



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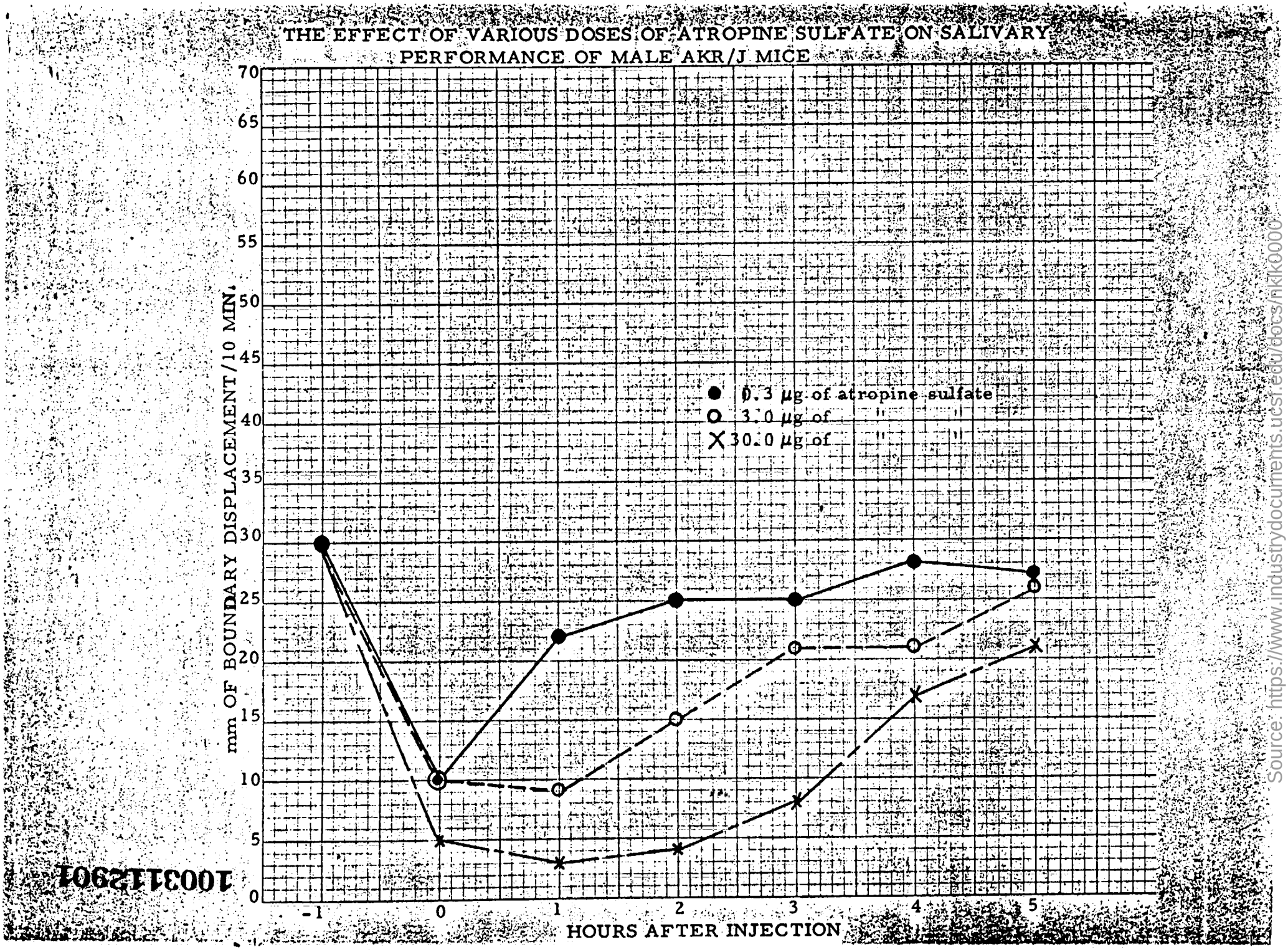

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